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I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on July 11, 2006, to the Group fax number: (571)273-8300 to the attention of Examiner Stacey Chen.

Shmuel Livnat

Atty Dkt: 29025.0001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:	Examiner: Stacy Chen
Arlene RAMSINGH *et al.*	Group Art Unit: 1648
Serial No.: 09/879,572	Atty Dkt: 29025.0001
Filing Date: June 12, 2001	Customer No. 30827
For: COXSACKIEVIRUS B4 EXPRESSION VECTORS AND USES THEREOF	

4TH DECLARATION OF ARLENE I. RAMSINGH PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, the undersigned, declare as follows:

1. I am a co-inventor of the present application. My *Curriculum Vitae* and professional background have been submitted as part of earlier Declarations. The comments below are in response to (a) the USPTO's requirement, appearing in the last two Office Actions, that the Coxsackievirus B4 (CB4) in the claims be deposited in a patent depository and (b) the PTO's position that practice of this invention is not enabled without a deposit as starting material. A number of the points made in a previous declaration[1] are reiterated here.

2. **AVAILABILITY OF THE STARTING VIRUS**

(a) Coxsackieviruses are picornaviruses. cDNA corresponding to the virus's entire genome can, in fact, be synthesized using standard chemical and biochemical techniques, without reliance on a "natural" template. (An example of such synthesis was described by Cello, J, Paul, AV and Wimmer, E (2002) Science *297*:1016-18, using methods and tools that were available at the time the present invention was made). Thus, armed with the sequence of the viral genome which is publicly available, a person wishing to practice the present

[1] 3rd Ramsingh Declaration Under Rule 132 ("Ramsingh-3") for a discussion of the history of the virus, its deposit in the ATCC, and its sequencing.

invention could start "from scratch" with a newly synthesized cDNA corresponding to the CB4 viral genome described in the present application and claims (*i.e.*, the variant designated CB4-P), transcribe the cDNA into RNA, insert the RNA into cells, and generate new virions with the corresponding RNA genome.

(b) CB4 was deposited with the ATCC by the New York State Department of Health in the early 1950's under a strain designation "JVB" (ATCC Accession # VR-184). This is the prototypical CB4 and as such, has been maintained, propagated and disseminated to the public by the ATCC for over 50 years. Eliminating the prototypical CB4 strain from its repository would contradict the ATCC's primary mission of preserving biological materials. A Board of Scientific Directors advises the ATCC on its scientific collection, so that any decision to discontinue a prototypical biological specimen such as the above CB4 would be met with substantial resistance from the scientific community including professional associations such as the powerful American Society of Microbiology and the American Society of Virology. (Consider, for example the pressure from the scientific community that has prevented the CDC from destroying repository strains of smallpox virus.)

(c) Unintentional loss of the deposited CB4 strain by the ATCC would be resolved by re-obtaining the strain from elsewhere, such as the New York State Department of Health, the original depositor. The ATCC could also obtain the CB4 strain from any investigator who has published on the virus since professional journals have instituted a requirement that materials described in publications be made available to the public. In the even more unlikely event that the ATCC ceases to exist or that CB4 is no longer available from them, the virus would remain available from the New York State Department of Health (original source), any researchers who have published on CB4 and public health laboratories that do surveillance studies. Under the "self-policing" system in place in the scientific community, reports that a researcher who has published on this virus fails to provide the virus to a requestor would result in a ban on subsequent publications by the refusing researcher. Thus, the virus is and will be available to the public.

3. **Availability and Significance of CB4-P Sequence Information**

(a) As already discussed in the record of this case, my laboratory sequenced half of the genome of CB4 ("half-sequence"; Ramsingh *et al.* 1992[2]) which sequence is also in GenBank as Accession #S39291[3]. This "half sequence" contains all of the information necessary to practice the claimed invention. The relevant sequences for making the VP1 vector (*e.g.*, claims 1, 3-12 and corresponding nucleic acid claims) are 2826-2834 and 2853-2861 and are included in the "half sequence". The relevant sequence for making the other type of claimed vector (*e.g.*, claims 13-15, 17-18) is ^{744}ATG GGA^{749}. As described, an insertion containing the cloning site and the 3C protease site is made after nucleotide 746.

(b) Both the CB4-P I have used and sequenced (from the N.Y. State Department of Health strain which was deposited at the ATCC) and the CB4 that was obtained later from the ATCC (from the same deposit)) and sequenced by Jenkins *et al.* [4] have the same sequence at positions 2826-2834, 2853-2861, and 744-749.

(c) Knowledge of the sequence of the "half genome" that I did not sequence is unnecessary and irrelevant to the practice of the invention because the VP1 vector relies on making insertions into a region encoding a defined T helper cell epitope of a viral structural protein. This other "half genome" encodes non-structural viral proteins that play a role in viral replication. These non-structural proteins are expressed and function in infected cells but are never packaged into viruses. Similarly, for the second type of vector we claim, insertions are upstream of the structural protein VP4.

(d) While the Examiner seems to think that CB4-P is "unique," no virologist would agree with this. How to best explain this would depend on what the Examiner thinks makes CB4-P unique. From a virologist's point of view, the CB4-P variant is a CB4 virus of the JVB strain. If I wanted to "make" CB4-P, the published half-sequence contains all of the information for the capsid proteins that make up the virus. As noted above, the remaining half sequence encodes proteins involved in viral replication. Therefore, the CB4-P half sequence I have published, together with the Jenkins *et al.*, published sequence of JVB CB4

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[2] Ramsingh *et al.* 1992. "Identification of candidate sequences that determine virulence in Coxsackievirus B4." *Virus Res 23*:281-292

[3] See http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=250908)

[4] Jenkins *et al.* 1987. "The complete nucleotide sequence of coxsackievirus B4 and its comparison to other members of the picornaviridae." *J. Gen. Virol. 68*:1835-1848)

(obtained from the ATCC) could be used as the basis for synthesizing a compete viral cDNA and actually creating the CB4-P virus (see Item 2(a) above). A virus made in this fashion would be identical to CB4-P. One would not be able to distinguish it by any serological assay. An assertion that a single nucleotide substitution in the region encoding the non-structural protein would make it a "different" virus runs counter to what is accepted by those in the field and ignores concepts of genetic relatedness. If one was to follow what I understand to be the PTO position in constructing a viral classification scheme, each variant of each strain of each viral serotype would have its own branch - there would be no relatedness and no clustering of variants, in opposition to scientific fact and consensus.

(e) Below are responses to statements made in the pending Office Action at page 9 (Examiner's response to Applicant's "Point 5"):

> *"The Office recognizes that changes in viral genomes take place from generation to generation as a result of selective pressures and mistakes in transcription and translation."*

Response: This appears to reflects a misunderstanding of the basic biology of these viruses. There is no transcription in CB4 - it is a plus-sense RNA virus. The genomic RNA itself is the transcript. Plus-sense RNA which is translated is also used as a template for the synthesis of negative-sense RNA strand which is the template for making multiple plus-sense RNA (genome) copies for incorporation into progeny virions.

> *"It is understood that a virus progeny from the original JVB virus would yield a virus that has a sequence that is not identical to the original JVB sequence. However, the specific claiming of CB4-P requires CB4-P. One cannot work with the designated strain unless it has been provided."*

Response: CB4-P is not a ***strain*** but is considered the same strain as the JVB strain of CB4.

> *"If the claims were drawn to viruses that are defined by features of CB4-P that are desired, such as certain mutations in various places in the genome, there would be not be a deposit issue. However, because Applicant has claimed the specific virus itself, the virus itself must be made publicly available."*

Response: CB4 (whether designated CB4-P or JVB strain) is publicly available. Any variant of the JVB strain of CB4, not just CB4-P, can be used to practice the claims, as discussed above and below, in view of the knowledge of sequence of CB4 viruses including that designated CB4-P (half-genome[2]), CB4-V (half-genome[2]) or CB4 (Jenkins *et al.*, full genome[4]).

(f) Below are responses to statements in the pending Office Action at page 12 (Examiner's response to Applicant's "Point 9"):

> *"In response to Points 8 and 9, it is acknowledged that the JVC strain is likely to perform similarly to the CB4-P virus. While this may be true, the fact remains that Applicant is claiming the CB4-P strain."*

Response: Again, the Office incorrectly characterizes CB4-P as a "strain." It s not a strain.

> *"Arguing that there are functional equivalents available does not compensate for the lack of availability of the claimed strain."*

Response: The claims are not directed to a "strain". As discussed above and elsewhere in the record, the virus *strain* is publicly available; alternatively, its genome can be synthesized and the virions re-produced using conventional methods.

> *"Information available from GenBank is not irrevocably fixed but is corrected and updated as additional sequence information becomes available. The Genbank accession number may refer to sequences which change after the application filing date."*

Response: Given all the published sequence information about CB4 (whether designated JVB or CB4-P), adequate information exists to enable the practice of the invention as claimed irrespective of the GenBank record. Moreover, to correct what appears to be a misapprehension by the Examiner, even if someone were to submit a new sequence for a virus with the same "name" to GenBank, it would be given a new record and not replace #S39291 which is based on a 14 year old publication. Even in cases where an investigator detects a sequencing or clerical error in his/her own submission and submits a correction, the original record is not wiped out; rather, new "correct" sequence is added so that the scientific community can compare the earlier and later submitted sequences.

(g) Below are responses to statements in the pending Office Action at page 10, 2nd paragraph (Examiner's response to Applicant's Points 6A/6B) that need to be addressed in the present context:

> *"There are acknowledged differences between JVB and CB4-P, and the viruses are claimed separately (see claims 2 vs 3), indicating that the two viral strains are not the same.*
>
> * * *
>
> *It is interesting to note that Dr. Ramsingh distinguishes between CB4-P and CB4-V, going so far as to rename JVB as CB4-P."*

Response: Again, these are not different viral strains. There was no difference between JVB and CB4-P. I could have just as well retained the name JVB, or put a "-P" after it. The "P"

in CB4-P stands for "prototype" strain. JVB is that prototype strain, so there would have been no sense in adding a "-P" after JVB. I wanted to distinguish JVB/CB4-P from the virulent variant which was referred to as CB4-V. For the sake of parallel naming, I therefore adopted the use of CB4-P to pair with CB4-V, leaving the JVB strain designation out. Note that CB4-V is a variant, but is nevertheless a virus of the JVB strain. As an accurate, though more unwieldy form of naming, I could have chosen JVB-CB4-P and JVB-CB4-V for the two variants of the JVB strain of CB4. I'm not sure what the Examiner means by stating that it is "interesting" that I went "so far" to rename JVB as CB4-P. My reasons are explained in the previous sentences. In the end, none of this changes the fact that JVB equals CB4-P.

As for the separate claiming of JVB and CB4-P, this was done by the original attorney who drafted the application and claims, and who did not focus on these strain/variant issues that are being discussed here. When reviewing the application, I, as a layperson with respect to patents, did not appreciate the meaning or significance of the fact that claim 2 which is in fact indistinguishable from claim 3, should not have been written as a separate claim. Any mention of "JVB" in the claims could have been omitted as extraneous because the intention was to direct the dependent claims to one strain of CB4 that was known originally as JVB and became referred to in my lab at a later stage as CB4-P. Thus Claim 2 (and claim 19) should have been omitted altogether. It would have been scientifically more accurate to have written claim 3 (in its current form that incorporates earlier amendments) as:

> *Claim 3. The recombinant attenuated coxsackievirus B4 virion of Claim 1 which is a virion of the strain designated CB4-P or JVB."*

Again, none of this changes the fact that that JVB and CB4-P are alternative names for the same viral strain and my basic points that there is no need for a deposit of CB4-P virus to enable practice of the claimed invention.

4, Discussion of Disclosed Cloning Strategies for Practicing the Invention

(a) Mutations and Viral Replication

(i) To reiterate, although RNA viruses lack a "proofreading" or "editing" mechanism (see Ramsingh-3) mutations that accumulate in the genome are generally point mutations that are either silent (same amino acid) or that result in an amino acid substitution. During replication, viral genomes that accumulate mutations in critical

sites will not give rise to infectious progeny and are lost from the population. For example, consider one round of viral replication. The virus enters a cell, the RNA genome is uncoated and replicates. One RNA template can be used to make multiple copies of RNA. If 1000 copies are made, a number of copies "*m*" will have mutations that, when packaged with the viral proteins will yield non-infectious virions. The remaining (1000 - *m*) copies, will be packaged with viral proteins and yield "infectious" (=replication-competent) virus. Only such viruses will continue on to the next cycle of replication. At the end of multiple rounds of replication when virus is harvested, only infectious virus is present. This virus population may exist as a collection of what virologists now call "quasi-species" because it contains viruses that may differ by a few mutations. Quasi-species that replicate well will dominate the population and quasi-species in which mutations occurred in critical sites will be defective and will comprise, at most, a small (and diminishing) subset. Upon additional cycles of replication, the defective quasi-species will be further diluted and the predominant quasi-species will be the one that replicates well, as if it were "wild type" (meaning unchanged) from the starting population with which the investigator began.

(ii) If a person wishing to practice the present invention starts with the disclosed CB4 (whether one prefers to call it CB4-P or JVB, which, as we have established in prior documents and here, are the same) and grow this virus multiple times over a 50-year period, the resulting progeny viruses are still CB4 (whether one prefers to call them CB4-P or JVB). Although mutations occur, the virus does not become a different (or unique) coxsackie virus such as CB3 or CB5 or some other new coxsackie virus. It remains CB4 (whether designated CB4-P or JVB). After 50 years, there will in all likelihood exist some point mutations by which the "ending" virus differs from the "starting" virus. Nonetheless, the virus is still CB4 (whether designated CB4-P or JVB). As noted above, the lack of a proofreading mechanism results in minor genetic variations, **not** the creation of some new virus that is unique and therefore "unavailable to the public unless deposited." Thus, a person who begins with CB4 virions or cDNA (whether designated CB4-P or JVB) has adequate

information in the patent application and the public domain to practice the claims fully.

(b) Practice of the Invention

One can start with CB4 (whether designated CB4-P or JVB). It does not matter which source of the CB4 virus one begins with, whether the CB4 distributed by ATCC, accession # VR-184, the CB4-P in my laboratory, or from any other source. The processes set forth in the application to produce the virions and nucleic acids of the present claims are fundamentally as follows:

(1) The first step involves constructing a full-length cDNA clone of the viral RNA genome (standard procedure).

(2) To make, for example, CB4/HIV recombinants expressing HIV sequences in the VP1 capsid (corresponding to claims 1, 3-12):

(i) Construct the VP1 vector as described in the application[5] by inserting the indicated restriction sites (exemplified as Stu 1 and a Nar 1 sites) at the specified positions. This engineering is done by PCR. **It does not matter** if the sequence of the entire starting CB4 genome is or is not exactly the same as that disclosed in the in specification.

(ii) Insert heterologous (*e.g.*, HIV) oligonucleotides into the VP1 vector at the Stu 1 and Nar 1 sites as described, creating "chimeric" or "recombinant" clones

(iii) Transfect cells with RNA transcripts of these chimeric/recombinant clones to give CB4/HIV chimeras/recombinants comprising (for example) the HIV sequences fused to the VP1 capsid protein.

(3) To make CB4/HIV recombinants expressing HIV sequences at that amino-terminus of the viral polyprotein (corresponding to claims 13-15, 17-18):

(i) Construct the polyprotein vector as described in the application[5]. Insert an appropriate cloning site (exemplified as the Mlu 1 cloning site) and a 3C protease recognition site upstream of VP4. Again, it does not matter if the sequence of the entire starting CB4 genome is or is not exactly the same as that disclosed in the in specification.[5]

(ii) Insert HIV sequences into the Mlu 1 site of the polyprotein vector as described in the application.

[5] Also in Halim *et al.*, 2000, *AIDS Res Hum Retrovir* 16:1551–1558

(iii) Transfect cells with RNA transcripts of these chimeric/recombinant clones to give CB4/HIV chimeras/recombinants comprising (for example) HIV sequences fused at the N-terminus of the viral polyprotein.

9. In conclusion, the CB4 virus or cDNA of the viral genome that can be the starting material for the claimed recombinant coxsackievirus B4 virions[6] (which are engineered to contain an inserted heterologous nucleic acid that encodes a heterologous polypeptide) are either publicly available or may be prepared synthetically based on information in the application and in the public domain, for example, in several published papers cited herein and in GenBank. Nothing further, beyond the deployment of conventional methods, is required to make and use the claimed virions and nucleic acid compositions. Given the above remarks, I can see no basis for a position that people in the field would not be able to practice the invention fully unless a patent deposit of "CB4-P" virus were made available.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under U.S.C. 18, § 1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

July 11, 2006 _____

Date Arlene I. Ramsingh

[6] and for the claimed nucleic acid molecules